Extraction and characterization of gelatine from chicken skin

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ABSTRACT

Gelatine is mixture of peptides and protein produce by partial hydrolysis of collagen from the animal skin, connective tissue and bones. Gelatine has gelling, foaming and emulsifying properties that contribute to a wide range of applications in the food, pharmaceutical, photographic and cosmetic industries. In the current study, gelatine was extracted from the chicken skin and was characterized in term of yield, molecular weight, melting point and viscosity. Two different pre-treatment methods using acetic acid and nitric acid were used during preparation of gelatine. The yield of gelatine using acetic acid and nitric acid pre-treatment are 11.19 %(w/w) and 9.18%(w/w) respectively based on dry weight basis. Both gelatines showed the same molecular weight pattern range from 53 to 250 kDa. The viscosity of gelatine using acetic acid and nitric acid pre-treatment are 3.3 mPa.s and 2.8 mPa.s respectively.

ABSTRAK

Gelatin adalah campuran peptida dan protein hasil daripada hidrolisis separa kolagen berasal dari kulit haiwan, tisu perantara dan tulang. Gelatin mempunyai sifat elastik, berbuih dan pengemulsi yang menyumbang kepada pelbagai aplikasi dalam makanan, farmaseutikal, industri fotografi dan kosmetik. Di dalam kajian ini, gelatin telah diekstrak daripada kulit ayam dan dicirikan dari segi hasil, berat molekul, takat lebur dan kelikatan. Dua kaedah yang berbeza dengan menggunakan asid asetik dan asid nitrik dikaji semasa penyediaan gelatin. Hasil gelatin menggunakan asid asetik dan asid nitrik sebagai rawatan memberi hasil 11.19% (w / w) dan 9.18% (w / w), masing-masing berasaskan berat kering. Manakala bagi ujian berat molekul gelatin, kedua-dua molekul gelatin menunjukkan corak berat molekul yang sama 53-250 kDa iaitu berada di dalam lingkungan berat molekul gelatin seperti di pasaran. Nilai kelikatan gelatin menggunakan asid asetik dan asid nitrik pra-rawatan adalah 3.3mPa.s dan 2.8 mPa.s.

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LIST OF SYMBOLS

^{0}C	: Degree celcius
%	: Percentage
BSE	: Bovine Spongiform Encephalopathy
BSA	: Bovine serum albumen
FMD	: Foot-and-Mouth Disease
g	: gram
GME	: gelatine manufactures of Europe
H_2SO_4	: sulphuric acid
hr	: hour
HCl	: hydrochloric acid
kDa	: kilodalton
kN	: Kilonewton
kg	: kilogram
l	: liter
lb	: pound
M	: molarity
mg	: milligram
ml	: milliliter
mm	: millimeter
NaCl	: sodium chloride
NaOH	: sodium hydroxide
Nm	: nanometer
Pa.s	: Pascal second
α	: Alpha
β	: Beta
γ	: Gamma
μ	: Micro
Tm	: melting temperature
UV	: ultraviolet
vs	: Versus
w/v	: Weight / volume
v/v	: Volume / volume

CHAPTER I

INTRODUCTION

1.1 Research background

Gelatine is mixture of peptides and protein produce by partial hydrolysis of collagen from the animal skin, connective tissue and bones. It is a translucent, colourless, brittle when dry, flavourless solid substance. Gelatine has unique properties as a gelling agent because it can form liquid and gel based on the temperature change. Gelatine will softens and form liquid when being heating and turn back into gel during cooling. This property was known as thermo reversible gel. The melting temperature for gelatine is below 35°C which is below human body temperature. This property make it unique in terms of its fit sensory aspects, especially flavour release that are need for some food industry (Baziwane and He, 2003; Boran and Regenstein, 2009; Choi and Regenstein, 2000). Other gelling agents such as starch, alginate, pectin, and agar are carbohydrates and their gels cannot melt below body temperature because have high melting temperatures (Williams, 2007).

Gelatine has been widely applied in food, pharmaceutical, photographic, and cosmetic industries (Karim and Bahat, 2009; Yang et al., 2007; Zhou and Regenstein, 2004). In food industry gelatine is used as ingredients to improve elasticity, consistency and stability of food like deserts, candies, bakery product, jellied meats, ice cream and dairy products. Gelatine also used as stabilizer to modify the taste of the food product. Gelatine is added to yogurt to reduce and increase firmness. Gelatine also recommended enhancing protein level in food stuffs and suitable in body-building foods. Different concentrations of gelatine would give a wide range of textures in food products. Gelatine is compatible with milk proteins and can improve the taste of cakes and marshmallow.

In pharmaceutical industry, it can be used for encapsulation, production of hard and soft capsules, wound dressing and emulsions (Djagny et al., 2001). In photographic application, gelatine is use for lighting equipment which is the colour gel use to change the beam colour. For cosmetic usage, gelatine can make as styling gel usually use by swimmer to hold their hair in place because gelatine not dissolve in cool water or pool. It also can be used in nail polish remover and make up application. Other than that, a lot of beauty product nowadays uses collagen in their product for whitening, repair skin damage and some good for repairing our tissue in body.

Alkali and acid treatment is required before the hydrolysis of collagen into gelatine. The function of alkali treatment is to remove non-collagenous proteins and pigment. Another function is to weakening the collagen structure leading to higher quality of gelatine. In most of the acid extraction process, citric acid is used because it does not change the texture of gelatine in term of colour or odour. Acid treatment will effectively removes odours and colour from the raw material (Boran and Regenstein, 2009; Zhang et al., 2007). There are two types of gelatine which is type-A and type-B gelatine. Type-A gelatine is produced from acid-treated collagen and type-B is produced from alkali-treated. Acidic treatment very suitable for less cross-linked collagens that usually use for pig skins whereas alkali treatment is used for more complex cross-linked sources such as bovine hides. Whether organic acid or inorganic acid can be used for extract collagen directly from animal tissue but the different is amount of collagen that can be extract and the quality of collagen produce. Examples of organic acid are acetic, citric and lactic acid (Sadowska et al., 2003). Hydrochloric is the example of inorganic acid that employed for extraction of collagen. However inorganic acid give worse performance compare to organic acid (Skierka and Sadowska, 2007).

1.2 Problem statement and motivation

Most of the available gelatines have been produced from mammalian resources, either pig skins or cowhides (Simon et al., 2002). Gelatine from mammalian sources such as from bovine and pig skins account for 46% of the world gelatine output, followed by bones and hooves, representing 23% and 29% of the total gelatine production, respectively and only the remaining percentage, i.e. 1% comes from marine sources (Gómez et al., 2002). Mammalian gelatine has been use because it have high melting, gelling point and it is thermo reversible (Gudmundsson, 2002). The cow bone is most preferred collagen source for producing high-quality gelatine (Rowlands and Burrows, 2000). Gelatine extraction from fish by-products are seldom used because they are mainly used for animal feed supplements due to their small size (Gildberg, 2002).

Traditional gelatine productions are manufactured from mammalian resources such as pork skin, cattle hides and cattle bones (Cho et al., 2005). Based to the report Gelatine Manufacture of Europe, 95% gelatine is made up from hide porcine and bovine and the rest from bones of porcine and bovine. Gelatine produce from pig skin cannot be used for some food due to aesthetic and religion objections (Judaism, Islam and Jews) for example. Muslims are prohibited to consume animals that are not properly slaughtered according to Syariah law. Therefore, gelatines that are produced from bovine source cannot be consumed by Muslims if the animals that are used to make those gelatines are not properly slaughtered according to Syariah law. But if beef gelatines are prepared based on religious requirement it accepted as a food additive (Badii and Howell, 2006). From that, the increases market for halal food has gained attention from both researchers and industry (Karim and Bhat, 2009).

However due to the outbreak disease BSE (bovine spongiform encephalopathy) known as mad cow disease and foot-and-mouth disease (FMD) commonly derived from mammalian part, the search from other sources of gelatine has been continuously investigate. Researchers are not only continually searching for an alternative to gelatine, and also to find new sources of gelatine. Within the past few years, there has been increased interest in the market in gelatine derived from fish and poultry. Poultry skin and bones are expected to yield gelatine in the near future, but commercial production is currently limited by low yields (Schrieber and Gareis, 2007).

Nowadays a lot of research has been conducted to make a gelatine from fish, however it has been limited application due to the gel formed is less stable and had a weak rheological properties compare to gelatine extracted from land mammals (Shahiri et al., 2010). However, there is too little research concerning production of gelatine from chicken skin. At present, the fish gelatine production is very low, yielding about 1% of the annual world gelatine production of 270,000 metric tonnes (Jamilah and Harvinder, 2002). Nowadays, there is great request for Halal products so chicken is the best selected sources for halal product. Furthermore, the production of gelatine from chicken skin can be beneficial to the food industries since most of the chicken in Malaysia is Halal certified. Therefore, the study of gelatine from chicken, such as skin is interest as sources of collagen to extract gelatine. Instead of being waste that can cause pollution to environment, chicken skin can be used for production of gelatine. The waste not only causes pollution but also it emit defensive odour (Takeshi and Nobutaka, 2000). Those gelatines can be used to replace gelatines that are produced from bovine and porcine sources.

1.3 Objective of the research

The main objective of this research is to extract and characterize the gelatine produced from chicken skin

1.4 Scopes of the research

In order to fulfil the research objective, the following scopes has been outlined.

i. To produce gelatine from chicken skin.
ii. To study the effect of, two different solutions which are acetic acid and nitric acid during the preparation step on the gelatine properties.
iii. To characterize the chicken derived gelatine in terms of molecular weight, melting point, yield and viscosity.

CHAPTER II

LITERATURE REVIEW

2.1 Collagen

Collagen is one of the most abundance proteins present in the bodies of mammals. Collagen is major dominant structure in the living body. It is tasteless and colourless solid substance derived from the fibrous protein collagen. About one half of total body made up of collagen. Collagen is mostly found in fibrous tissues such as tendons, ligaments and skin (collagen), and is also abundant in corneas, cartilages, bones, blood vessels, the gut, and intervertebral discs (Brinckmann et al., 2005). Collagen is one of the key structural proteins found in the extracellular matrices of many connective tissues in mammals; the whole-body protein making up about 25% to 35% of content (Muyonga et al., 2004).

Collagen played an important role to support the body structure of animal. It connects and supports other body tissues such as skin, bone, tendons, muscles, connective tissue and cartilage. It also supports the internal organs and is even present in teeth. Collagen works strongly elastin in supporting the body’s tissues (Madison, 2011). Even the blood vessels depend on both collagen and elastin. It works hand-in-hand with elastin in supporting the body’s tissues. This combination of collagen and elastin is very important in many parts of the body including lungs, bones, and tendons. It also supports the internal organs and is even present in teeth. Basically, it gives body tissues form and provides firmness and strength.

Collagen molecules are arranged with a 35-nm gap between molecules head-to-tail and are found in larger structures having staggered bundles, that is, adjacent collagen molecules are not aligned with each other (Gutsmann et al., 2003). Charged and uncharged residues are found to be periodically clustered along the sequence of collagen at about every 230 residues, which is around 67 nm, although this distance may vary somewhat among different tissue sources of collagen (Holmes et al., 2001). The ending fibril can be from 20 to 400 nm in diameter and is stabilized by four covalent cross-links per collagen molecule, two at either end of the molecule. This suggests that the collagen molecules are aligned such that the maximum electrostatic and hydrophobic interactions occur between different molecules as shown in Figure 2.1.

Collagen is generally considered as incomplete protein since the concentration of some essential amino acids is low in collagen and consequently, in gelatine (Belitz et al., 2004; Nelson and Cox, 2005). Therefore, gelatine is mixture of fractions composed entirely of amino acids joined by peptide linkages to form polymers that have molecular mass from 15,000 to 400,000 and not a single chemical entity.

Collagen can be extracted from pig, bovine, fish and chicken. Different sources of collagen will result in different physical properties. However, in the industry, the main sources of the collagen are become limited to those that obtained from pigs and bovine skin and bones (Takeshi et al., 2002). Collagen from mammals for example bovine and pig is different than collagen extracted from fish. The properties of collagen markedly vary with the habitat, species, and part of fish being isolated (Falguni et al., 2010).

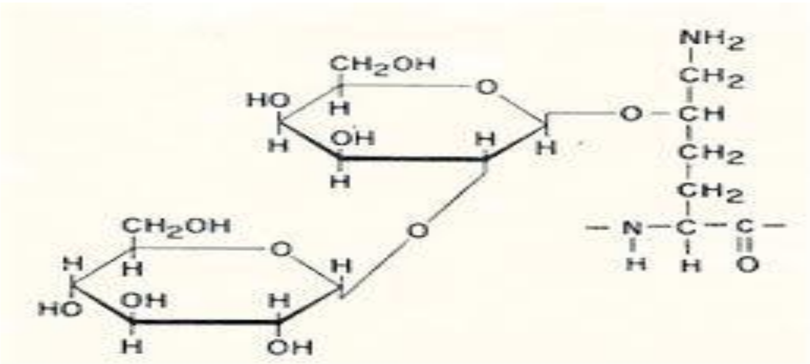

Figure 2-1: Structure of collagen

Treatment process such as alkali and acid treatment will be subjected for the collagen from by-product of land animal. After all the process, the structure of collagen will break down and the product produce is known as gelatine. The processes involve in the production of gelatine for commercial manufacturing of gelatine are extraction, filtration and clarification, evaporation, sterilization, drying, grinding and sifting, and storage (Gomez-Gullien et al., 2009).

There exist at least fourteen general types of collagen. The most familiar type I, the predominant genetic type that consist of three polypeptides chain. Two chains identical are call α1: the third beings call α2. Type I collagen is the type occurs widely, primarily in connective tissue such as skin, bone, and tendons. Usually collagen type I is widely used in food industries, cosmetic, pharmaceutical, biomedical, and tissue engineering due to its excellent biocompatibility and biodegradability (Liu et al., 2010). Whereas collagen types I, II, III, and V are called fibril- forming collagens and have large sections of homologous sequences independent of species, among which first three types are known to be chemotactic (Chevallay and Herbage, 2000). Type II is the type of collagen occurs practically exclusively in cartilage tissue. Then type III are strongly dependent on age: very young skin can contain up to 50%, but in the course of time this is reduced to 5–10% (Gelse et al., 2003). For type IV collagen, being present in basement membrane, the regions with the triple-helical conformation are interrupted with large non-helical domains, as well as with the short non-helical peptide disturbance. Other types of collagen are present in very low amounts only and mostly organ-specific (Schrieber and Gareis, 2007).

Collagens molecules from which gelatine are derived are composed of three α-chains intertwined in the so-called collagen triple helix. This particular structure is due to the almost continuous repeat of the (Gly-X-Y) sequence and each chain is generally more than 1000 residue long. Glycine is the most abundant acid in gelatine which is X and Y mostly proline and hydroxyproline. Usually every molecules contain two or even three different α chains, described as α 1, α 2 and α 3, with the difference lying in the amino acids present in X and Y positions of the triplets. About 25% of dry gelatine contains proline and hydoxyproline that stabilize its structure (Russell et al., 2007). This triplet of amino acids allows collagen chains to twist into a helical structure. Each collagen molecule contains 3 chains twisted around each other to form a triple helix as shown in Figure 2.2.

Collagen comprises a triple helix structure issue which forms fibres, arranged in bundle, which make up of connective matrix. The triple helix structure is stabilized by intra-chain hydrogen bonds and all the main chain N-H and C = O groups are involved in these types of interactions. The triple helix gives collagen a rigid structure. It maintains the mechanical integrity of tissues. Less amino acid content should result in a less statically hindered helix and may affect the dynamic properties of gelatine.

The size of triple-helix is about 300 nm in length, and the chain has a molecular weight of approximately 105 kDa (Papon et al., 2007). When process of acid or alkaline hydrolysis, a mild derivative process occurs and the fibrous structure of collagen is broken down irreversibly due to the rupture of covalent bonds. Denaturation of soluble collagen due to the breakdown of hydrogen and probably electrostatic bonds in hot water (40 °C) takes place by destroying the triple helical structure of collagen to produce one, two or three random chain gelatine molecules that give a solution in water of high viscosity. It will destabilize the triple helix by means of a helix to coil transition and leading to conversion into soluble gelatine (Gomez-Guillen et al., 2005).

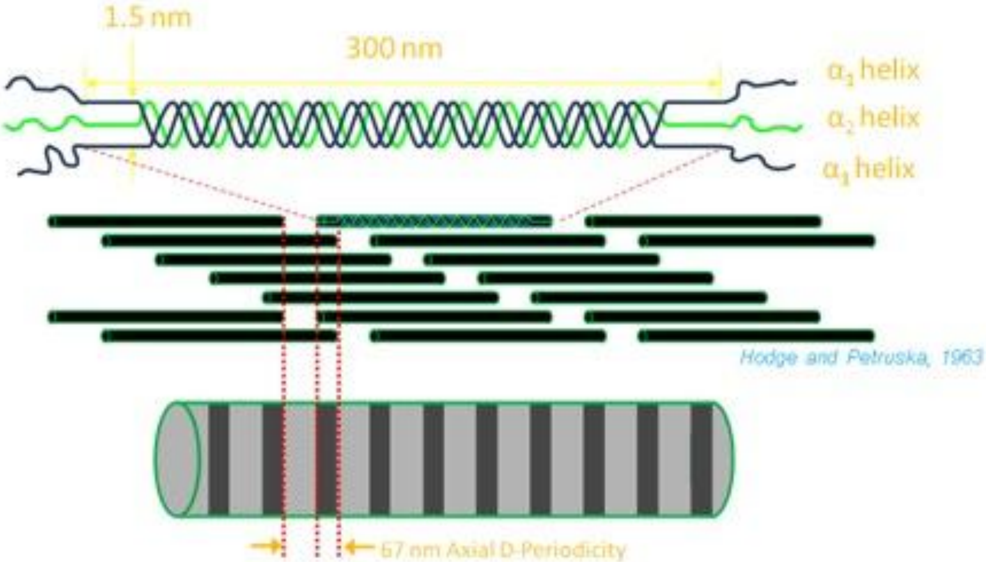

Figure 2-2: Schematic diagram for collagen molecule

2.2 Gelatine

Gelatine is the product of thermal denaturation of insoluble collagen by partial hydrolysis process with various molecular weights (MWs) and isoionic points (IEPs) (Gomez-Gullien et al., 2009). Collagen denaturation causes separation of rods and total or partial separation of the chain (Papon et al. 2007). This is because of destruction of hydrogen bonds, causing loss of the triple helix conformation, and following denaturation, the polymers exist in a coiled form. During the process of gelatine, raw animal material is treated with dilute acid or alkali, resulting in partial cleavage of the crosslinks: the structure is broken down to such an extent that ‘‘warm-water-soluble collagen’’, then gelatine is formed (Schrieber and Gareis, 2007).

The degree of crosslinking in gelatine is highly variable. It depends on collagen type, tissue, animal species and also age. The properties and gelling abilities of gelatine, involving a partial denaturation of denatured collagen molecules depend on all these parameters since gelatine is derived from denatured collagen (Gomez-Gullien et al., 2009). The properties of the resulting gelatines are greatly influenced by the two main factors that are the initial collagen characteristics and the precise treatment process. The properties of the resulting gelatine are influenced by the source and type of collagen (Binsi et al., 2009).

In many aspects the chemical composition of gelatine are similar, to its parent molecule. However gelatine is not composed of one size of collagen fraction or peptide chain but is a combination of many fractions varying in size, including the whole α -chain of the tropocollagen molecule (a trimmer of around 330 kDa that aggregates to form the larger collagen structures) and hydrolytic fragments of parts of the α -chains of different lengths. Gelatine is a mixture of different polypeptide chains including α-chains, β (dimers of α-chain) and γ (trimmers of α-chain) components with a molar mass of around 90, 180 and 300 $\times 10^3$ g/mol, in aqueous solutions (Rbii et al., 2011). Higher gel strength is showed by gelatine which contains more α-chains. Therefore, all the processing steps of this gelatine should avoid extensive degradation of peptide structure in order to obtain high gelling strength (Liu et al., 2008). The properties of the resulting gelatine are depending on the sources and type of collagen (Binsi et al., 2009).

2.3 Application of gelatine

Gelatine, one of the most popular biopolymers, is widely used in food, pharmaceutical, cosmetic, and photographic applications because of its unique functional and technological properties. The most common application of gelatine is used as a jellying agent. In sugar jellies industries, gelatine will give the gel and delay crystallisation of the sugar in the jellies. Recent years, the gelatine is added with Arabic gum in the production of tougher jellies. (Boran et al., 2010; Gómez-Guillén et al., 2011; Kittiphattanabawon et al., 2010). Another function of gelatine is to reduce the moisture content in sugar jellies.

Gelatine is a water-loving material which act as hydrophilic properties and can absorb up to ten times its weight in water (GMAP, 2011). Thermally reversible gels with water are formed from an aqueous solution of a few per cent gelatines and the gel-melting temperature (<35°C) which is below body temperature, which gives gelatine products unique organoleptic properties and flavour release. Due to the thermo reversibility properties, this process gives the gelatine gel its unique “melt-in-mouth” quality (Boran et al., 2010). Gelling agents other than gelatine sources such as starch, alginate, pectin, agar and carrageenan are all polysaccharides from plant sources, but their gels are lack of the melt-in-mouth and elastic properties of gelatine gels.

Gelatine is one of component the most accepted biopolymers and is extensively utilized in food because of its unique functional and technologies properties (Karim and Bhat (2009). Gelatine has been used as a beverage clarifier a fining agent for white wine, as a beer clarifier and to clarify fruit and vegetable juice especially to clarify apple juice and pear juice. Moreover, gelatine also utilized in confections mainly for providing chewiness, texture, and foam stabilization. It is low-fat spreads to provide creaminess, fat reduction, and mouth feel. Gelatine is a dairy product to provide stabilization. In ice cream, stabilizer is used to prevent the formation of coarse ice crystals and gelatine was the easiest stabilizer used. It also decreases the rate of melting, give body and a firm smooth texture and baked goods to provide emulsification, gelling, and stabilization.

Gelatine, being low in calories, is normally recommended for use in foodstuffs to enhance protein levels, and is especially useful in body-building foods. In addition, gelatine is also used to reduce carbohydrate levels in foods formulated for diabetic patients (Gilsenan and Ross-Murphy, 2000). Nutritionally, both collagen and gelatine are low quality of protein that can improve quality of skin and finger nails (Meler, 2006). Skin is made up of collagen and as our age increase, production of collagen drops off and skin sags because it get thinner, weaker and less resilient. This is automatic related to amino acid content. There are specific amino acids content in skin's structure such as glycine, proline, hydroproline and alanine decrease with age and bad diet (King 2011).

In the pharmaceutical industry, gelatine is widely used for the manufacture of hard and soft capsules, plasma expanders, and in wound care. Karim and Bhat (2009) also suggested that gelatine with low melting point could be used in dry products for microencapsulation. Gelatine also been used as a matrix for implants, in inject table drug delivery microspheres, and in intravenous infusions. In fact collagen has already found significant usage in clinical medicine over the past few years, such as injectable collagen for repair tissue defects, haemostasis, burn and wound dressings, hernia repair, bioprostetic heart valves, vascular grafts, a drug –delivery system, ocular surfaces, and nerve regeneration (Lee et al., 2001). There are also reports in which live attenuated viral vaccines used for immunization against measles, mumps, rubella, Japanese encephalitis; rabies, diphtheria, and tetanus toxin contain gelatine as a stabilizer (Gimenez et al., 2005). Gelatine can form fibres with extra strength and stability by self-aggregation and cross-linking, which makes it useful in drug delivery systems (Lee et al., 2001).

In cosmetic and health care products, gelatine is used as a gelling ingredient in face creams, body lotions, shampoos, hair sprays, sun screens and bath salts and bubbles. The types of fish are influence the pharmaceutical application for example Codfish gelatine are used for evaluation of allergen city of commercial and food-grade fish gelatine (Hansen et al., 2004). For Pacific codfish skins the application in pharmaceutical is to investigation of changes of antioxidant activity in skin tissue and the arrangement of collagen fibres using ultraviolet radiation induced skin photo aging (Hou et al., 2009).

Collagen and health benefits related with it have led to establishment of collagen-supplement industry. Nowadays collagen supplement are meant to mainly improve skin appearance and being image-obsessed society and got high demand (Jamie 2009). Field of sport nutrition is another area that increasing worldwide demand for hydrolysed collagen. Collagen can automatically boost lean muscle gain, decrease recovery time, rebuild damage joint structure without surgery and improve cardiovascular performance on athletes. Therefore, a lot of athletes and body builders use hydrolysed collagen as clean sources for muscle gain, tendon and ligament repair, fast recovery time and maximum performance (King 2011).

In photographic industry, gelatines are needs for film coating, colour paper, graphical and X films, and printer ink. The unique chemical and physical properties of gelatine make it an important component in the photographic industry. Gelatine serves many useful purposes in the preparation of silver halide emulsions in the production of photographic film. Such gelatines have been reported to have a good film formation and emulsifying properties (Schrieber and Gareis, 2007).

2.4 Properties of gelatine

There are a lot of properties effects the quality of gelatine for example physical attributes and chemical characteristic. Physical attributes include gel strength, viscosity, melting and gelling temperature. The quality of gelatine is measured by the gel strength or Bloom value, including low (<150), medium (150–220) to high Bloom (220–300); commercially, high viscosity gelatine is preferred and fetches a higher price. The chemical properties of gelatine are affected by amino acid composition, molecular weight distribution and triple helix formation (Gomez-Guillen et al., 2002). Amino acid composition is similar to that of the parent collagen, thus influence by animal’s species, breed, age, manner of feeding the animal, storage conditions of raw materials and type of tissues. The differences in molecular weight distribution were also affected its chemical properties which result from the variation in the nature or extraction conditions (Zhou and Regenstein, 2006).

The source and type of collagen also influence the properties of the gelatine (Binsi et al., 2009). The principal raw materials used in gelatine production are cattle bones, cattle hides, and pork skins but mostly from pig. Other than this source, there are alternative raw material that can be used in gelatine production, including by-product from chicken and fish processing industries. For production of large amount high-quality of gelatine, fish skins have received lot of attention from researcher as alternative raw material. Therefore, studies on various species of fish skin gelatine have been a famous research for the production of high quality gelatine.

One of the ways to improve gelatine is by manipulating the characteristics of gelatine by addition of salts. Fish gelatine properties can be modified through addition of enhancer like salts, glycerol, variation of pH and in combination of other ingredient such as sucrose (Koli et al., 2011; Sarebia et al., 2002). Saline ion will cause the collagen to interact with water molecules and folding indirectly. In addition, when the fish skins have been washing using NaCl and KCI at 0.8 M, it will result in a higher gelling ability and stability on fish gelatine (Gimenez et al., 2005). Choi and Regenstein (2000) also stated that melting point of gelatine decreased directly as the concentration of NaCl went up to 14%. NaCl is very sensitive to fish gelatine because the concentration NaCl is able to break both of hydrophobic and hydrogen bonds. Thus preventing the stabilization of the gel junction sites, either by prevent hydrogen bond formation or by modify the structure of liquid water.

Other than amino acids, properties of gelatine also contain moisture, ash, calcium, copper and iron. The moisture content of gelatine is different at different pH. The moisture content is increasing if the pH is increasing (Chen et al., 2007; Fishman et al., 2000). However the composition such as ash, calcium, copper, and iron must be in lower amount in gelatine. This is because the composition will give low quality of gelatine. For example, if more than 2 ppm of iron content in gelatine, it will show grey strain on food product. In addition, colour of gelatine also depends on the raw material extracted (Pan et al., 2003). The official standard of good quality of gelatine is to be free of objectionable taste or offensive odour and colour.

Except from amino acid composition, other factors such as functional properties of are gelatine also influenced by the distribution of the molecular weights and compositions of its subunits. An important factor affecting the quality of fish gelatine is the environmental condition of the fish species. Generally, collagen and gelatine, prepared from low temperature fish species contain lower amounts of proline and hydroxyproline, lower number of hydrogen bonds and have a lower melting point than species from a higher temperature environment. During gelatine process, the conversion of collagen to gelatine yields molecules of varying mass, due to the cleavage of inter-chain covalent crosslinks and the unfavourable breakage of some intra-chain peptide linkages (Zhou et al., 2006).

The properties of gelatine are strongly depend strongly to pH in the reaction mixture and on the charge balance (determined by the gelatine pectin ratio), which will influence the degree of electrostatic associations and ionic interactions in the gelling system (Farris et al., 2009). Not only species or tissue from which it extracted are influenced the physicochemical properties, but also by the severity of the manufacturing method (Gilsenan and Ross-Murphy, 2000).

An optimization of the tissue extraction procedures and a better knowledge of the properties of fish-skin gelatine could be helpful in extraction of gelatine from fish (Gomez-Guillen et al., 2002). Based on research, fish gelatine has known limited application because the gels formed tend to be less stable and to have worse rheological properties compare to gelatines from land mammals (Shahiri et al., 2010). These limitations because of gelatine in cold water fish contain less proline than in warm blooded animals.

2.5 Amino acid

Gelatine usually contains 90% protein, 18 types of amino acids and 7 essential for people to consume (Ali, 2010). The high quality of gelatine are contains high protein, low ash and heavy metal, small molecular weight, easy absorption and utilization, high biological value, promoting absorption of vitamin and mineral. Table 2-1 show the comparisons of amino acid content in gelatine derive from several type's fish such as Cod skin, Alaska Pollock skin, Megrim and Tilapia skin compare to pork skin.

Table 2-1: Composition of amino acid in fish skin

Amino acid	Cod skin[a]	Pollock skin[b]	Megrim[a]	Tilapia skin[c]	Pork skin
Alanine	96	108	123	123	112
Arginine	56	51	54	47	49
Aspartic acid	52	51	48	48	46
Cysteine	0	0	-	0	0
Glutamic acid	78	74	72	69	72
Glycine	344	358	350	347	330
Histidine	8	8	8	6	4
Hydroxylysine	6	6	5	8	6
Hydroxiproline	50	55	60	79	91
Isoleucine	11	11	8	8	10
Leusicine	22	20	21	23	24
Lysine	29	26	27	25	27
Methionine	17	16	13	9	4
Phenylalanine	16	12	14	13	14
Proline	106	95	115	119	132
Serine	64	63	41	35	35
Theorinine	25	25	20	24	18
Tryptopthan	0	0	-	0	0
Tryrosine	3	3	3	2	3
Valine	18	18	18	15	26

Reference: [a] Gomez et al., (2000), [b] Zhou et al., (2006),[c] Sarabia et al,.(2000)

Amino acid composition will affect chemical properties of gelatine which is similar to that of the parent collagen, thus influence by animal's species and type of tissues (Zhou and Regenstein, 2006). In gelatine, all the amino acids are present except tryptophan and have low in methionine, cystine and tyrosine due to the degradation during hydrolysis (Jamilah and Harvinder, 2002). Although some differences in amino acid composition are apparent across collagens derived from different sources, there are certain features that are common to and uniquely characteristic of all collagens.

There are only mammalian gelatine contain large amounts of hydroxyproline and hydroxyl sine, and the total amino acid (proline and hydroxyproline) content is high (Gilsenangher and Ross-Murphy, 2000). The high amino acid content in gelatines from mammalian and warm water fish is considered to be related to a lower critical concentration and higher melting point. Secondly a higher molecular weight MW (300 kDa) gelatine is known to have a higher Bloom value than low MW gelatine.

Thermal stability of amino acid content was reported to have a major influence in the collagen (Prabjeet et al., 2011 and Falgani et al., 2010). There is a well-known almost linear relationship between the hydroxyproline content and the denaturation temperature of the collagen. When hydroxyproline content is lower the denaturation temperature also lowers (Hickman et al., 2000). As the amount of hydroxyproline contain increases, rheological properties and gel strength of gelatine also increase (Prabjeet et al., 2011).

The composition of amino acids is of particular importance regarding both gelatine gel strength and melting point (Badii and Howell, 2005). Proline and hydoxyproline will be influenced by the raw material of gelatine used. Gelatine from warm-blooded and from warm water fish give have higher collagen compare to cold-water fish because contains of higher amino acid and increase proline and hydroxyproline. Although less imino acid contain in cold-water fish compare to warm water fish and mammalians, the contents of amino acid, molecular weight and gelatine viscosity maybe will be higher contain for cold-water fish (Gómez-Guillén et al., 2002; Gudmundsson, 2002). High content of hydrophobic amino acid have similar effect to gelatine even though it is less prominent (Badii and Howell, 2005).